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Assessment of Oxidative Stress in *Peste des petits ruminants* (*Ovine rinderpest*) Affected Goats

A. K. Kataria* & N. Kataria
Apex Centre for Animal Disease Investigation, Monitoring and Surveillance
College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences,
Bikaner – 334 001, Rajasthan, India
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ABSTRACT

The aim of the present investigation was to evaluate oxidative stress in goats affected with *peste des petits ruminants* (PPR). The experiment was designed to collect blood samples from PPR affected as well as healthy goats during a series of PPR outbreaks which occurred during February to April 2012 in different districts of Rajasthan state (India). Out of total 202 goats of various age groups and of both the sexes, 155 goats were PPR affected and 47 were healthy. Oxidative stress was evaluated by determining various serum biomarkers *viz.* vitamin A, vitamin C, vitamin E, glutathione, catalase, superoxide dismutase, glutathione reductase and xanthine oxidase, the mean values of which were 1.71±0.09 µmol L^{-1}, 13.02±0.14 µmol L^{-1}, 2.22±0.09 µmol L^{-1}, 3.03±0.07 µmol L^{-1}, 135.12±8.10 kU L^{-1}, 289.13±8.00 kU L^{-1}, 6.11± 0.06 kU L^{-1} and 98.12±3.12 mU L^{-1}, respectively. Each parameter analysis of variance showed highly significant effect (P=0.0001) of health status and age category. Further interaction between health status and age category was also highly significant (P=0.0001) for each parameter studied. The results indicated that vitamins A, C, E and glutathione levels depressed by 18.95%, 38.67%, 47.64%, and 47.39%, respectively and the serum catalase, superoxide dismutase, glutathione reductase and xanthine oxidase activities increased by 90.79%, 75.11%, 90.34%, and 44.06%, respectively in affected animals as compared to that in healthy ones. On the basis of the altered levels of serum biomarkers of oxidative stress it was concluded that the animals affected with PPR developed oxidative stress.

Key words: goats, oxidative stress, Peste des petits ruminants, serum biomarkers

ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi stres oksidatif pada kambing yang disebabkan oleh *peste des petits ruminants* (PPR). Penelitian dilakukan dengan mengumpulkan sampel darah kambing yang terkena PPR dan kambing sehat selama terjadi wabah PPR dari bulan Februari hingga April 2012 pada berbagai bagian wilayah Rajasthan (India). Total sampel kambing adalah 202 ekor, meliputi berbagai kelompok umur dan jenis kelamin, yang terdiri atas 155 ekor kambing yang terkena PPR dan 47 ekor merupakan kambing sehat. Stres oksidatif dievaluasi melalui penentuan berbagai biomarker serum, yaitu vitamin A, vitamin C, vitamin E, glutation, katalase, superoksida dismutase, glutation reduktase, dan xantin oksidase, dengan nilai tengah secara berurutan 1,71±0,09 µmol l^{-1}, 13,02±0,14 µmol l^{-1}, 2,22±0,09 µmol l^{-1}, 3,03±0,07 µmol l^{-1}, 135,12±8,10 kU l^{-1}, 289,13±8,00 kU l^{-1}, 6,11±0,06 kU l^{-1} and 98,12±3,12 mU l^{-1}. Hasilnya menunjukkan bahwa vitamin A, C, E, dan glutation menurun 18,95%; 38,67%; 47,64%; dan 47,39% secara berurutan. Aktivitas katalase, superoksida dismutase, glutation reduktase, dan xantin oksidase meningkat 90,79%; 75,11%; 90,34%; dan 44,06%, secara berurutan pada ternak sakit dibandingkan dengan ternak sehat. Berdasarkan perubahan taraf biomarker stres oksidatif pada serum maka dapat disimpulkan bahwa ternak yang terkena PPR mengalami stres oksidatif.

Kata kunci: kambing, stres oksidatif, Peste des petits ruminants, biomarker serum

*Corresponding author:
A.K. Kataria
125, Kailashpuri, Bikaner 334 001, Rajasthan, India.
Phone: 0091-151- 2546399, Fax: 0091-151-2549348
E-mail: akkataria1@rediffmail.com

INTRODUCTION

Peste des petits ruminants (PPR), also known as ovine rinderpest and pneumoenteritis complex, is an *office international des epizooties* list A disease of sheep and goats caused by morbilli virus and characterized by high morbidity and high mortality rates resulting in considerable economical losses. Its important clinical signs include pyrexia, necrotic stomatitis, catarrhal inflammation of the ocular and nasal mucosa, enteritis and pneumonia (Nisbet *et al*., 2007). The productivity of PPR affected animals is depressed in association with marked immune suppression (Kataria *et al.,* 2007).

Stress management for the affected animals is essential for prognosis and protection of other animals. Limited available scientific reports have focused on increased oxidative stress as an underlying etiopathogenesis of this disease (Gil *et al*., 2004), which needs to be elucidated further. Oxidative stress plays an important role in ruminant medicine. Redoxhomeostasis is involved in many physiological functions. When the fine balance between prooxidative processes and antioxidative system is disturbed oxidative stress occurs.

Ruminant animals undergo several periods of severe challenge of the antioxidative system. Research in oxidative stress has been associated in various pathological processes in veterinary medicine (Kataria *et al*., 2010a); however, very little emphasis has been given to infectious disease including PPR in small ruminants. Looking towards paucity of work associating oxidative stress in pathogenesis of PPR in goats, the present investigation was carried out with the aim to understand variations in the endogenous serum antioxidants (vitamin A, C and E, and glutathione) and enzymes (catalase, superoxide dismutase, glutathione reductase, and xanthine oxidase).

MATERIALS AND METHODS

Blood samples were collected from affected (n= 155) and healthy (n= 47) goats at varying ages and both sexes during February to April 2012 in different districts of Rajasthan state (India). The animals were then subgrouped by the age: young (90 PPR affected and 25 healthy) and adults (65 PPR affected and 22 healthy). The disease was diagnosed on the basis of classical clinical signs, post-mortem findings and confirmed in laboratory by c-ELISA using sera from animals recovered from the disease. The goats were of *Sirohi* and *Marwari* breeds and belonged to private farmers kept at organized farms or as unorganized flocks. All the animals were free from blood protozoan parasites and gastrointestinal parasites.

Blood was collected through jugular vein directly into additive-free sterilized tubes for measurements of serum biomarkers including antioxidants (vitamin A, vitamin C, vitamin E and glutathione) and enzymes (catalase, superoxide dismutase, glutathione reductase and xanthine oxidase) (Kataria *et al*., 2012).

Data were subjected to group mean comparison (Kaps & Lamberson, 2004). Data analysis was carried out by 2-way ANOVA, including main effects of health status and age category as well as interaction between health status and age category.

RESULTS AND DISCUSSION

The mean ± SEM values of serum antioxidants and enzymes of oxidative stress in healthy and goats affected with *peste des petits ruminants* are presented in Table 1 and Table 2, respectively.

Serum Antioxidants

The health status showed variations in the levels of all the serum antioxidants *viz*. vitamins A, C, E and glutathione which depressed by 18.95%, 38.67%, 47.64%, and 47.39%, respectively in PPR affected goats as compared to healthy ones. Age category also showed variations in the levels of all the serum antioxidants in healthy as well as PPR affected animals. In each case adult animal showed higher levels as compared to young goats.

Inflammatory processes result in formation of free radicals which target lipids, DNA and proteins. Antioxidants provide defense mechanisms to detoxify radicals or repair oxidized molecules. Vitamin A, vitamin C, vitamin E, and glutathione are considered nonenzymatic antioxidants (Bose *et al*., 2012). Low levels of serum antioxidants in PPR affected animals indicate their depletion in an attempt to combat free radicals (Kataria *et al.,* 2010a) because Vitamin A along with vitamin C and E are considered as effective endogenous antioxidants that help the animals to combat free radicals generated during the process of oxidative stress (Kataria *et al*., 2012). Vitamin A has an effect on lipid peroxidation (Padayatty *et al*., 2003). Vitamin C works as an antioxidant because ascorbate free radical reacts poorly with oxygen and hence superoxide is not created. Instead two semidehydroascorbate radicals will react and form one ascorbate and one dehydroxy ascorbate. With the help of glutathione, dehydroxyascorbate is converted back to ascorbate (Kataria *et al*., 2010c). Vitamin E stops the production of reactive oxygen species during oxidation of fat. It protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. The oxidised α-tocopheroxyl radicals produced in this process are recycled back to the active reduced form through reduction by other antioxidants like ascorbate and retinol. Vitamin E is known as strong endogenous antioxidant (Kataria *et al*., 2010c). Glutathione is also considered as potent endogenous antioxidant. Scientists have correlated its depletion with the development of oxidative stress (Bernabucci *et al*., 2005). Glutathione spares ascorbate and improves antioxidant capacity of blood (Gropper *et al*., 2004).

Serum Enzymes

The activity of serum enzymes *viz.* catalase, superoxide dismutase, glutathione reductase and xanthine oxidase increased by 90.79%, 75.11%, 90.34%, and 44.06%, respectively in PPR affected animals as compared to that

Table 1. Mean±SEM values of serum antioxidants of oxidative stress in healthy and goats affected with *peste des petits ruminants*

Group	Antioxidants (μmol L^{-1})			
	Vitamin A	Vitamin C	Vitamin E	Glutathione
Affected (155)	1.71±0.09	13.02±0.14	2.22±0.09	3.03±0.07
Young (90)	1.12±0.07[a]	19.00±0.11[c]	1.44±0.07[a]	2.06±0.06[a]
Adult (65)	2.30±0.09[c]	23.47±0.11[d]	3.00±0.09[b]	4.00±0.07[c]
Healthy (47)	2.11±0.09	21.23±0.11	4.24±0.09	5.76±0.11
Young (25)	1.71±0.08[b]	11.04±0.10[a]	3.48±0.09[c]	3.78±0.11[b]
Adult (22)	2.51±0.09[d]	14.00±0.14[b]	5.00±0.09[d]	7.74±0.11[d]
ANOVA (P values)				
Health status (HS)	0.0001	0.0001	0.0001	0.0001
Age Category (AC)	0.0001	0.0001	0.0001	0.0001
HS x AC	0.0001	0.0001	0.0001	0.0001

Note: Figures in the parentheses indicate the number of animals
Means in the same column with different superscript differ significantly (P=0.0001)

in healthy ones. In healthy group, adult animals showed higher mean values than young animals for each parameter. In PPR affected group the trend reversed, where young animals showed higher mean values than adult animals. This showed that young animals were affected greatly in comparison to adult animals. Increased activities of all the enzymes reflected the endogenous response of the animals to offer defense against oxidative stress.

Catalase is present in all living organisms and functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al.,* 2004). Hydrogen peroxide is a harmful by product of many normal metabolic processes to prevent damage and hence it must be quickly converted into other less dangerous substances. Higher serum catalase could be due to higher rate of formation of hydrogen peroxide (Kataria *et al.,* 2010b) indicating higher oxidative stress. Barski & Spodniewska (2012) have also related increased erythrocytic catalase activity in rats to the existence of strong oxidative stress.

Superoxide dismutase is responsible for the quenching of superoxide radicals which are released during the chemical reactions of the various metabolic pathways. It catalyses the dismutation of superoxide into oxygen and hydrogen peroxide. This enzyme is an important constituent of endogenous antioxidant defense system. Higher levels of serum SOD indicate oxidative stress (Kataria *et al.,* 2010c). It is considered as the key antioxidant enzyme and known to reverse fibrosis, perhaps through reversion of myofibroblasts back to fibroblasts (Vozenin-Brotons *et al.*, 2001).

Glutathione reductase is an enzyme that reduces glutathione disulfide to the sulfhydryl form which is an important cellular antioxidant. The higher activity of glutathione reductase is used as an indicator for oxida-

Table 2. Mean ± SEM values of serum enzymes of oxidative stress in healthy and goats affected with *peste des petits ruminants*

Group	Serum enzymes			
	Catalase kU L^{-1}	Superoxide dismutase kU L^{-1}	Glutathione reductase kU L^{-1}	Xanthine oxidase mU L^{-1}
Affected (155)	135.12± 8.10	289.13± 8.00	6.11±0.06	98.12±3.12
Young (90)	170.00± 8.20[d]	318.26± 7.00[d]	7.00±0.07[d]	115.00±3.33[d]
Adult (65)	100.24± 8.30[c]	260.00± 9.00[c]	5.22±0.05[c]	81.24±1.10[b]
Healthy (47)	70.82±10.12	165.11±13.00	3.21±0.12	68.11±1.91
Young (25)	48.84± 8.70[a]	130.00±12.88[a]	2.00±0.12[a]	49.22±1.99[a]
Adult (22)	92.80±14.28[b]	200.22±10.00[b]	4.42±0.12[b]	87.00±1.59[c]
ANOVA (P value)				
Health status (HS)	0.0001	0.0001	0.0001	0.0001
Age category (AC)	0.0001	0.0001	0.0001	0.0001
HS x AC	0.0001	0.0001	0.0001	0.0001

Note: Figures in the parentheses indicate the number of animals
Means in the same column with different superscript differ significantly (P=0.0001)

tive stress in animals (Kataria *et al.,* 2010c). Increased glutathione reductase activity indicated scavenging of reactive oxygen species (Villegas *et al*., 2012).

Xanthine oxidase is a form of xanthine oxidoreductase that generates reactive oxygen species (Ardan *et al.,* 2004). It is not a scavenger of free radical but along with free radical scavengers it can be used as a marker of oxidative stress. Its involvement in several inflammatory and oxidative stress-related diseases is documented (Vida *et al.,* 2011). It plays an important role in the catabolism of purines and catalyses the conversion of hypoxanthine to xanthine and that of xanthine to uric acid, which are the last steps in purine metabolism. The byproduct of these reactions is a toxic superoxide radical. This reaction is considered as potential source of oxygen free radical (Zhang *et al.,* 2010). The uric acid product from xanthine oxidase catalysis contributes to the antioxidant capacity of the blood. In stressed animals higher serum xanthine oxidase may indicate oxidative stress (Kataria *et al*., 2010c).

Depressed status of serum antioxidants and higher status of serum enzymes in PPR affected animals were recorded which indicated development of oxidative stress in these animals. Earlier researchers had also reported alterations in the levels of antioxidant enzymes in PPR affected sheep (Nisbet *et al.,* 2007). Oxidative events against PPR infection are not well elucidated in animals. Organisms have several enzymatic and non-enzymatic antioxidant systems that overwhelm harmful effects of free radicals. Under certain conditions, antioxidants mechanisms are impaired and free radicals are increased and antioxidant mechanisms may become insufficient to prevent oxidative damage completely. Consequently, oxidative stress develops. Free radicals can cause protein oxidation, lipid peroxidation and DNA damage (Kocyigit *et al*., 2005). There has been growing interest in the role of antioxidant function in controlling inflammatory disease states and inflammation is one of the important symptoms of PPR (Kataria *et al.,* 2007). Inflammatory processes are associated with generation of increased number of free radicals. Persistent oxidative stress induced by inflammatory processes is a self-perpetuating process and cause progressive accumulation of DNA damage in target organs (Bartsch & Nair, 2006). Effects of immunosuppression on pathogenesis of *peste des petits ruminants* virus infection have been reported (Jagtap *et al*., 2012). Immunosuppression leads to generation of reactive oxygen species (Kedzierska *et al*., 2011).

Oxidative stress due to an increase in reactive oxygen species or a deficiency in antioxidant defense mechanisms causes structural and functional modifications of lipid, protein and DNA-containing macromolecules of the cell. This supports the view that PPR infection causes oxidative stress and consequently lipid peroxidation and the by-products may be used as biomarkers of damage in various tissues (Nisbet *et al.,* 2007). The results clearly reflected development of oxidative stress due to PPR in goats and magnitude was greater in young animals in comparison to that in adult ones.

CONCLUSION

Peste des petits ruminants (PPR) affected animals experienced strong oxidative stress. The periodic assessment of oxidative status in ruminants is necessary to enhance body defence system for diseases, including PPR.

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